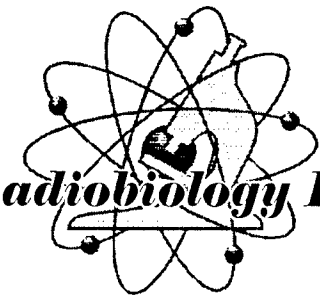

Armed Forces Radiobiology Research Institute

Animal Studies of Residual Hematopoietic and Immune System Injury From Low Dose/Low Dose Rate Radiation and Heavy Metals

Central Research Institute of Roentgenology
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Preface

In recent years, the focus of much radiobiological research has shifted from the biological and health effects of prompt, high dose radiation to those of low dose and low dose rate exposures. Although there are still victims of criticality accidents and industrial accidents (e.g., Chernobyl) who receive high doses of radiation over a relatively short period of time, there are thousands of radiation workers, as well as persons exposed to environmental releases of radiation, whose health may become affected owing to chronic or repeated exposures to lower doses and dose rates. These exposure levels may be below the threshold to cause immediate or short-term symptoms such as accompany acute radiation syndrome, but high enough to cause problems such as anemia, preleukemic states, myeloproliferative disorders, and other effects even several years after exposure may have been terminated. Examples of such groups include those affected by the fallout near Chernobyl, those living near rivers (especially the Techa river in the southern Urals region of Russia, and to a much lesser extent, the Columbia near Hanford, Washington) wherein fission products were released, workers involved in reactor or weapons construction, and perhaps astronauts/cosmonauts exposed to space irradiation.

It is clear that there is an adaptive response to low dose rate exposures. However, as the authors of this report describe, recovery of marrow precursor cells after a second exposure may be incomplete. This could be due to any combination of factors such as persistent deficiency of stem cells, accelerated cell cycling of marrow precursors leading to increased ratios of S-phase populations among stem cells, shortened lifespans of immature erythroid and myeloid cells, and others. Part of the problem in studying the effects of damage repair after low dose exposures is that the damage is minimal, and detection of changes accordingly is difficult. More research needs to be carried out in this field.

Researchers in both the Western countries and the countries of the former Soviet Union and Eastern bloc have been looking into these and similar problems. The results reported, from their work and that of others cited by the authors, are consistent with those found at Argonne and other DOE laboratories. Some of the findings in rodent work were surprising, however, showing significantly higher lethality rates at low dose rates of 50–100 mGy/day than we would have expected. (The lethality rates in the control populations were also above those normally seen in U.S. laboratories; this may be partially responsible for the results.)

One unique feature of this report is the combination of radiation effects with those from the heavy metals cadmium and lead. These metals, both marrow-toxic and immunotoxic in their own right, affect the outcomes of radiation exposures even at low

doses and low dose rates. Particularly in situations involving radiation contamination from occupational accidents or environmental releases, one may expect to seldom encounter victims adversely affected by radiation alone. A host of other physicochemical agents, ranging from metals such as these to solvents and other substances, are likely to be present. The authors' findings regarding the additive and/or synergistic effects of simultaneous or nearly simultaneous exposure to radiation and toxic metals are important for this reason. We trust that readers of this work will find their approach and findings stimulating and useful for their own understanding and efforts.

Grateful acknowledgment is given to Dr. Tom Seed of AFRRI for reviewing the preliminary reports and offering valuable scientific advice. The careful and thorough editorial support of Jane K. Myers is also greatly appreciated.

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Contents

Introduction

Humans and animals are constantly exposed to background amounts of electromagnetic (e.g., ionizing) radiation as well as chemical toxicants from the environment. The main problem is to determine the exposure levels that are not injury producing over the long term.

It is widely known that acute, high-dose ionizing radiation has long-term effects on blood and immune cell populations, especially blood stem cells and committed precursors of hemopoiesis, as evidenced by lifelong studies of A-bomb survivors from Hiroshima and Nagasaki, for whom increased risk of certain leukemias is well documented. Meanwhile, few studies have examined the effects of low dose rate (LDR) and continuous irradiation.

As shown in animal experiments, radiation damage of cell populations proceeds, mainly, by induction of DNA lesions and, to a lesser degree, by altering cell membranes. These molecular rearrangements (strand breaks, base modifications, etc.) are mostly repaired by well-specified enzymatic systems. Nonrepairable lesions may be detected later as chromosome aberrations and gene mutations. DNA repair *in vivo* has been extensively studied only for acute irradiation regimens. Similar studies following LDR irradiation are very scarce, because of the minimal damage detected after low dose treatment, even in quite radiosensitive tissues [1-4]. From these studies, one may deduce that total counts of bone marrow and peripheral blood were not suppressed below normal limits after continuous irradiation with dose rates under 0.2 cGy/d. However, less evident changes of blood/immune cell populations (i.e., genetic mutations and cytokinetic parameters) could be detected if more sensitive biomarkers were used.

Impaired self-renewal of hemopoietic cells and functional deficiency of the growth-supporting microenvironment are suggested after prolonged treatment at high dose rates [5,6]. The long-term consequences of low dose irradiation may include either severe anemia with evident features of preleukemia or evolving myeloproliferative disease, as shown in experiments with dogs [7]. In these animals, the rates of DNA repair after test doses of ultraviolet (UV) radiation were significantly altered compared to controls [8]. Moreover, LDR radiation causes activation of the DNA repair system, thus decreasing the rate of chromosome damage in hemopoietic tissues, as shown in chronically irradiated mice [9]. This acquired radioresistance is referred to as an adaptive response.

Hence, the long-term effects of low dose/low dose rate irradiation seem largely dependent on the capacity of irradiated radiosensitive tissues to repair DNA damage induced at a given daily dose. These effects are, however, marginally expressed, and the damage is comparable to the toxic effects induced by other environmental toxicants. In addition to background radiation, many inhabitants of big cities, industrial workers, and farmers are subject to complex effects of toxic heavy metals and some organic compounds from the environment. Both radiation and some environmental pollutants increase the relative risk of blood and immune disturbances, including leukemias [10,11].

The biological actions of toxic environmental mixtures may vary over a broad range with respect to prevalence of distinct harmful factors and duration of exposure [12, 13]. Some data indicate acute effects from naturally occurring toxic mixtures on blood and immune cells, correlating with the amount of heavy metals contained in the mixture [14].

Complex blood pathology is observed after combined treatment with radiation and toxic chemicals [15,16]. However, different toxic chemicals may either increase or decrease radiation damage. Hence, the summary effects of a given toxic mixture upon the test organism depend upon the combination of harmful factors and the resistance of target cell populations.

Hence, some basic combined effects of chronic irradiation and toxic metal ions are worthy of study and evaluation in long-term animal experiments. Such data could be extrapolated to human studies in industrial and environmental medicine, concerning, for example, workers in nuclear plants as well as service staff of waste disposal sites and some categories of miners [17,18].

Lead and cadmium were chosen for combined radiation/

metal treatment in our experimental studies [19-21] because of their proven hematotoxicity and immunotoxicity. Lead ions are well known to inhibit hemoglobin synthesis in bone marrow, along with macrophage depression [22, 23]. Chronic treatment with cadmium salts causes hemodepression and decreased lymphocyte proliferation [24, 25] Moreover, Cd ions are able to mediate production of DNA-damaging radical species, thus suggesting their carcinogenic effects [26].

The effects of external, low dose irradiation upon the blood and immune systems of experimental animals have been studied at several centers: Institute of Biophysics, USSR Public Health Ministry, Moscow; Institute of Medical and Biological Problems, Moscow; Central Research Institute of Roentgenology and Radiology, St. Petersburg; Institute of Biophysics, USSR Academy of Sciences, Pushchino; Institute of Radiation Hygiene, Russian Public Health Ministry, St. Petersburg; and Institute of Experimental Pathology and Therapy, Sukhumi. For some other details, see table 1.

Low dose/low dose rate animal experiments in Russia were initially intended to assess hygienic allowances for chronic radiation exposure in humans working with low-intensity radiation sources. Based on the earliest rodent data, some estimates of probable "safe" doses in humans were derived [1,2]. It was presumed that the blood-damaging treatment of rats with 5 cGy/d corresponds to 1 cGy/d (360 cGy/year) for humans. Meanwhile, the effect of 0.2 cGy/d (72 cGy/year) irradiation on blood cells of humans may correspond to 1 cGy/d in rats and thus be able to induce the initial signs of irreversible blood pathology.

Among other related research, an extensive chronic experiment with dogs was undertaken for assessment of radiation effects during long-term space flight (reviewed [3]). Recent studies concerned possible responses of murine hemopoietic cells to chronic LDR treatment and rates of blood and immune system recovery [4]. Some experiments related to the combined toxicity of chronic irradiation and heavy metal uptake as assessed by posttreatment recovery rates [20,21].

Table 1. Characteristics of the most important long-term irradiation experiments performed at Russian research centers

Animal species	Dose rate cGy/d	Total dose Gy	Period of studies	Cells/functions studied after irradiation	Reference
Mice	4.3 cont	4.1	18 mo	Lymphocyte populations	27
Mice	1-6 mf	2	18 mo 6 mo	Marrow and peripheral blood morphology, CFUs	4
Mice	1-6 mf	< 10	12-18 mo 6 mo	Marrow morphology	28
Rats	0.2-5 cont	0.7-18	24 mo	Marrow and blood morphology, aberrant mitoses in marrow	1,2
Rats	0.2-1.7 cont	0.7-20	14-16 mo	Peripheral blood morphology	29
Rats	5-80	0.12-4.0	2 d-18 mo	Marrow and blood morphology, marrow cell cycle by flow cytometry	20, 31-33
Rats	5 + Cd or Pb	4.0	2 d-18 mo	Marrow and blood morphology	20,21
Dogs	0.06-0.34 cont	1.1-9.4	lifelong 72 mo	Marrow morphology and cytokinetics, immunological functions	3,34
Monkeys	1-4.9 mf	0.84-25.0	lifelong	Marrow morphology, chromosome aberrations	35

cont, continuous irradiation; mf, multifractionated regimen

Blood and Immune System Pathology Under Chronic Irradiation

Dose and Time Dependence

Direct damage of LD/LDR irradiation to blood cells, in terms of cell counts and cell viability, is illustrated in table 2.

Altered Blood Cytokinetics

Chronic radiation-induced changes of blood cytokinetics have been assessed, mainly, for dogs and mice [3,4]. Shortened cell cycle was shown for both erythroid and

Table 2. Features of blood cell damage induced by LD/LDR irradiation of animals

Type of pathology	Animal species	Effective DRs cGy/d	Period of irradiation d	Trend of change	Reference
White blood cell counts	Rats	5	30-60	Decrease	1,2
	Rats	1.8	30	Decrease	36
White blood cell counts, monocytes, eosinophils, band forms	Rats	5	15-60	Increase	37
Platelet counts	Rats	5	> 300	Decrease	2
Platelet counts	Rats	5	> 40	Decrease	38
Red blood cell counts	Rats	5	> 700	Decrease in RBC + reticulocytosis	1,2
Different marrow counts	Rats	5	> 30	Decrease of immature erythroid and myeloid forms	1,2
Total marrow counts	CBA mice	1-6	30-150	Decrease	4
Marrow counts	Same model	> 6	> 200	Myeloid cell decrease	4
Marrow counts	Dogs	0.3	> 300	Decrease in myeloid forms	39
Marrow CFUs	CBA mice	1-6	5-10	Decrease	4
Cycling CFUs	Same	> 3	20-30	Increase	4
Spleen CFUs	Same	1-6	28-100	Decrease	4
Granulocyte viability	Dogs	> 0.3	> 1000	Decrease	3
	Rats	5.0	> 60	Decrease	37
Other parameters:					
Ultraviolet fluorescence (leukocytes)	Dogs	> 0.3	> 60	Increase	40

myeloid precursors, accompanied by erythroid expansion in bone marrow. However, peripheral red blood cell (RBC) counts remained constant in irradiated dogs. Meanwhile, accelerated cycling of myelomonocytic cells was even accompanied by a moderate decrease in the peripheral polymorphonuclear leukocyte (PMN) counts. Thus, increased proliferation of the myeloid pool is not sufficient to support normal granulocyte counts in chronically irradiated animals [34].

Immunopathology

Table 3 illustrates the most common alterations of immune cell populations and appropriate defense reactions in chronically irradiated animals.

Table 3. Alteration of immune cell populations in chronically irradiated animals

Type of pathology	Animal species	Effective DRs cGy/d	Time at detection, d	Trend of changes	Reference
Lymphocyte scores (blood)	Rats	5.0	> 100	Decrease	1,2
Lymphocyte lysis *in vivo*	Rats	0.8-1.7	> 500	Increase	41
Lymphocytes (spleen, thymus)	CBA mice	1-6	> 30	Decrease	4
Spleen antibody-producing cells	Same model	4.3	> 5	Decrease	42
Lymph node antibody-producing cells	Dogs	> 0.3	> 800	Increase	43
Lymphocytes, mitogenic response	Dogs	> 0.2	> 300	Increase	43
Macrophage phagocytosis and natural killer activity	CBA mice	0.002 + incorp. nuclides	180	Decrease	44
Macrophage IL-1 and tumor necrosis factor production	Same model	0.002 + nuclides	180	Increase	44
Local microflora	Dogs	> 0.3	> 1000	Increase	3
Phagocytic ability of granulocytes	Dogs	> 0.3	> 300	Phasic	3
Autoantibodies in serum	Dogs	> 0.3	> 250	Increase	45

Postradiation Recovery of the Blood and Immune Systems

Postradiation changes of peripheral blood counts, if present in experimental animals, returned to control levels within several months [3,4]. However, subsequent recovery of marrow precursors and mature cells proved to be incomplete, as evidenced by deficient response to challenge stimuli (acute hemorrhage or gamma irradiation).

Decreased regeneration capacity was observed for both myeloid and erythroid cell pools [3,35]. Several factors may account for deficient hematological recovery after chronic irradiation:

- Persisting postradiation deficiency of hematopoietic stem cells observed after > 1 cGy/d for several months [4].
- Accelerated cell cycling of marrow precursors in irradiated animals, thus leading to increased ratios of S-phase populations among stem cells (colony-forming units [CFUs]) and committed marrow precursors [34].
- Shortened lifespan of immature erythroid and myeloid cells due to decreased cell viability in irradiated animals [3].

In the above experiments, the cell cycle distribution of bone marrow populations was assayed by means of autoradiography with 3H-thymidine or by microscopic analysis of cellular DNA contents using fluorescent DNA staining. Altered marrow cytokinetics proved to be a valuable parameter of radiation effects, thus necessitating repeated experiments with LD/LDR irradiation employing more efficient techniques, such as DNA flow cytometry.

Hence, our experiments were performed with white rats subjected to 5 to 80 cGy/d for a total dose of 0.12 to 4.0 Gy. The aim of the study was to trace blood and bone marrow changes after completion of chronic irradiation [20,30-33].

Age-Dependent Physiological Changes of Blood Parameters in Untreated Rats

Peripheral Blood and Bone Marrow Cell Counts

Longitudinal observations of animals, both untreated and radiation exposed, were performed from 1988 through 1993, thus allowing us to evaluate circadian and age-associated changes of blood cell populations.

Cellular composition of bone marrow and peripheral blood showed substantial changes with age (3 to 21 mo). Untreated animals exhibited a linear decrease of peripheral blood lymphocyte scores ($n = 6.9 - 0.19t$ [where t = time in mo]; $p < 0.05$), concomitant with increased granulocyte amounts. The number of peripheral erythrocytes tended to decrease in aging animals, however, accompanied by a distinct increase in RBC hemoglobin contents.

When analyzing bone marrow smears, the sum of myeloid (granulocytic) cells tends to grow during physiological aging, mainly due to the increased fraction of maturating cells ($n = 35.2 + 0.79t$; $p < 0.05$). Moreover, an age-associated drop in immature erythroid cell numbers was found in the bone marrow.

The amounts of pluripotent hemopoietic cells (CFUs) in marrow samples were evaluated in the model of irradiated mice as rat bone marrow recipients [46]. It was shown that this important index expressed a linear decrease with age ($n = 38.0 - 1.6t$; $p < 0.05$).

Hence, the process of normal aging in rats was accompanied by generally decreased blood cell production, along with limited rates of stem cell proliferation.

Age-Associated Alterations of Functional White Blood Cell Parameters

To assess the functional abilities of peripheral leukocytes,

their phagocytic and adherent capacities were determined in the untreated rats at different ages. The phagocytic population showed substantial age-dependent changes (table 4). For example, the percentage of zymosan-engulfing peripheral granulocytes increased significantly until the end of observations (up to 21 mo). However, it was accompanied by decreased numbers of NBT-positive phagocytes. The adherent population of leukocytes remained relatively constant with age except for a substantial decrease at 15 months.

Table 4. Age-dependent changes in phagocytic ability of granulocytes and leukocyte adherence in control rats

Parameters tested	Age of animals (mo) 3	9	15	21
Phagocytes, (% of total) + granulocytes	73.6±1.5	78.3±2.0 p < 0.05	79.2±1.5 p < 0.01	81.5±3.0 p < 0.05
NBT-positive granulocytes, %	45.0±3.1	40.6±2.2	32.1±1.9 p < 0.01	27.8±3.3 p < 0.01
Adherent leukocytes, (% of total)	16.1±1.8	18.2±1.6	9.5±0.9 p < 0.01	15.4±5.9
Number of animals	15	30	30	8

P values are shown for significant differences from 3-mo values.

Circadian Changes in Marrow Cell Cycling

Cell cycle distribution (CCD) in the bone marrow of untreated rats was studied every year, at fixed times (between February 1 and February 5). In total, 310 animals of similar age (3-4 mo) were subjected to this study. The cumulated results for several years are presented in table 5.

For these experimental series, certain circadian oscillations were found in the index of bone marrow cell cycling. The fraction of S+G_2+M cells began increasing in the morning, reaching its maximum by 1500 to 1800, followed by a decrease which approached nadir values around 0600 [20]. Throughout 1989-91, this parameter reached a maximum between 1500 and 2100, as compared to average levels. By

Table 5. Circadian changes in the size of the cycling fraction among bone marrow cells of normal rats

Time of day	Fraction of S+G_2+M myelokaryocytes, % of total (number of animals studied) 1989	1990	1991
12.00	16.3±0.5 (10) p < 0.05	18.6±1.0 (10) p < 0.05	- -
15.00	17.5±0.7 (10) p < 0.01	30.3±1.9 (10) p < 0.001	22.2±0.7 (33) p < 0.01
18.00	20.1±1.0 (10) p < 0.001	25.5±1.4 (10) p < 0.05	- -
21.00	14.6±0.6 (10) p > 0.05	28.9±1.0 (10) p < 0.001	24.2±1.8 (19) p < 0.05
24.00	13.5±0.4 (10) p < 0.01	24.1±2.0 (10) p > 0.05	- -
03.00	12.2±0.5 (10) p < 0.001	19.6±1.5 (10) p > 0.05	15.6±1.1 (17) p < 0.001
06.00	11.3±0.4 (10) p < 0.001	11.7±2.3 (10) p < 0.001	- -
09.00	14.4±0.7 (10) p > 0.05	15.8±2.5 (10) p < 0.05	12.6±1.7 (15) p < 0.001
12.00	16.0±0.7 (10) p > 0.05	17.7±1.4 (10) p < 0.05	- -
Average	15.1±0.3 (90)	21.4±0.8 (90)	19.6±0.4 (84)
	1992	1993	
12.00	16.7 ± 0.6 (36)	16.0 ± 0.9 (10)	

P values shown for difference from average.

contrast, the below-average values of S+G_2+M cells were observed from 0300 to 0900. These oscillations of marrow proliferative activity did, in fact, coincide with results obtained by the metaphase analysis approach [47].

Hence, the studies performed until 1993 showed that the size of the S+G_2+M cell fraction in normal rat marrow is most reproducible when the sampling is performed at noon

(at least for young rats aged 3-4 mo). Therefore, this time was routinely used for blood and marrow examinations in trials with radiation or metal treatment [20].

Circadian changes in the mean value of each component of rat myelogram and of the proliferative pool of bone marrow cells were also obtained [47]. These changes (table 6) were imposed on a regular decrease in the subpopulations of granulocytes or on a regular increase in the subpopulations of erythroid cells and lymphocytes (from 1200 to 0900 next day). This process was not influenced by the elimination of lymphocytes from the individual myelograms. These myelogram changes appeared to be due to the circadian mature-cell production rhythm and flows of the lymphocytes and the mature granulocytes between the bone marrow and the peripheral blood.

These data were compared to those obtained in the study of mitotic activity of the bone marrow in cancer patients [48]. It was found that, even at an early stage, tumors affected the circadian rhythm of bone marrow proliferation, reducing the amplitude of oscillation. A model simulating formation of the circadian rhythm of the bone marrow was suggested based on the possibility of arresting cells at the end of the G_1 phase. The rate of transition of G_1 cells to S phase was determined not only by endogenous "set-points" of the rhythm, which formed the basic wave of proliferation, but also by conditions of animal upkeep.

Seasonal and Age-Related Effects Upon Cell Cycle Distribution in Normal Rats

Data obtained at various seasons of the year were analyzed for untreated animals of different ages. The overall percentage values of noncycling and proliferating bone marrow cells were as follows:

Table 6. Cellular composition of untreated rat bone marrow, February 2-5, 1990 (circadian changes)

Type of cells	Time of day							
	12	15	18	21	0	3	6	9
Myeloblasts, promyelocytes, and myelocytes	21.3 ±2.2	21.0 ±2.3	18.9 ±3.0	19.8 ±2.7	20.3 ±1.7	19.5 ±2.7	17.1 ±1.5	17.7 ±2.0
Metamyeloid cells	11.4 ±2.5	11.2 ±1.4	8.6 ±1.5	7.4 ±1.0	12.3 ±1.6	9.9 ±1.2	7.7 ±0.9	9.1 ±1.4
Neutrophils	44.4 ±5.2	50.7 ±3.8	47.0 ±3.5	45.9 ±2.7	37.5 ±3.0	37.8 ±5.9	44.6 ±2.4	45.6 ±4.0
Erythroblasts, normoblasts	11.2 ±2.7	10.9 ±2.7	11.9 ±1.5	14.2 ±1.6	16.7 ±2.3	13.0 ±2.2	16.2 ±1.8	15.7 ±2.5
Oxyphilic normoblasts	11.5 ±3.6	5.0 ±1.3	8.2 ±1.4	12.8 ±2.0	10.4 ±2.9	13.3 ±4.1	10.4 ±2.6	11.1 ±2.6
Proliferative pool of bone marrow cells	32.5 ±3.9	31.9 ±3.2	31.0 ±3.4	33.6 ±3.5	37.1 ±2.6	32.8 ±3.5	34.3 ±2.0	35.6 ±3.4
Proliferative pool of myeloid cells	27.7 ±2.8	25.3 ±2.8	24.6 ±2.9	26.9 ±3.4	29.0 ±1.6	27.1 ±3.6	24.6 ±1.9	25.0 ±3.0
Proliferative pool of erythroid cells	51.3 ±6.8	67.1 ±6.3	59.5 ±5.2	53.3 ±4.1	64.6 ±5.2	54.4 ±5.9	63.8 ±6.5	59.9 ±3.8

$G_1 = 81.6 \pm 0.1\%$; $S = 9.6 \pm 0.1\%$; $G_2 + M = 8.8 \pm 0.1\%$

However, the average ratios of these subpopulations were significantly different when experimental results were subdivided according to season of sampling and age of the animals (table 7). The percentage of myelokaryocytes in $S+G_2+M$ phases was higher in the older age group compared to the younger animals ($p < 0.001$).

Table 7. Seasonal changes of bone marrow cell cycle distribution in rats of different ages

Season	N of rats	Age (mo)	P	Percentage of cells in $S+G_2+M$ phases	P
Winter	103	3.1±0.1		17.8±0.3	
	89	14.0±0.5	< 0.001	19.4±0.3	< 0.01
Spring	99	3.8±0.1		16.6±0.2	
	127	14.7±0.3	< 0.001	19.0±0.3	< 0.001
Summer	86	5.4±0.1		17.6±0.3	
	22	14.2±0.8	< 0.001	18.9±0.7	> 0.05
Autumn	65	5.7±0.2		20.2±0.4	
	43	10.5±0.4	< 0.001	19.5±1.1	> 0.05
Total	353	4.6±0.1		17.9±0.2	
	281	13.8±0.2	< 0.001	19.2±0.2	< 0.001

Along with this finding, distinct season-related changes of this parameter were noted in the younger groups. For this age group, the spring values of the $S+G_2+M$ fraction were significantly below the average ($p < 0.001$), and higher values ($p < 0.001$) were observed for the autumn experiments. Meanwhile, the number of cycling cells remained high in the senior group, and they were not dependent on the season. Hence, significant season dependence was established for CCD values of myelokaryocytes from young untreated rats, thus arguing for longitudinal oscillatory dynamics of marrow cell proliferation.

Changes in Cellularity and Cell-Cycle Distribution of Rat Myelokaryocytes

Peripheral Blood Parameters and Bone Marrow Patterns

The studies of common hematological parameters in peripheral blood did not reveal any significant deviations in RBC numbers nor white blood cell (WBC) contents and differential counts at various times (2 d to 18 mo) after chronic irradiation at total doses of 2 or 4 Gy.

Total marrow cellularity in irradiated animals did not differ. Differential counts showed an increased percentage of immature erythroid cells immediately after completion of 4 Gy irradiation, as compared to age-matched controls (16.8 ± 1.3 and 10.9 ± 1.2%, respectively). Similar changes persisted for 2 mo, fading away sometime later. All other indexes of bone marrow remained within control levels during the entire experimental period (up to 18 mo).

Time Dynamics of Bone Marrow CCDs

A special study was devoted to the long-term alterations of the bone marrow proliferative state after chronic irradiation. Systematic studies of CCD in total myelokaryocyte populations were performed, for example, after protracted irradiation at a TD of 4 Gy (DR = 5 cGy/d), followed by flow cytometry of cellular DNA.

Total cumulated doses applied were 2.0 or 4.0 Gy at DRs of 5 cGy/d (Series I, 1989-91), and 10 cGy/d (Series II, 1992-93). In both series, a total of 345 rats was employed, including those irradiated with TDs of 4.0 Gy (n = 155; this included 16 challenged with an additional 1.0 Gy) and 2.0 Gy (n = 69; this included 16 challenged with an additional 1.0 Gy) and untreated controls (n = 121). The animals were observed daily, and lethal cases were recorded.

At 6 mo after the end of radiation treatment, the cumulated lethality rates for group I (TD = 4 Gy) proved to be 15.7%, thus significantly exceeding those of group II (TD = 2 Gy) and control values (4.2 and 5.0%, respectively). Comparable rates after 12 mo of observations were 45.1, 33.3, and 19.1%. During 15 mo, 47% of rats died after a TD of 2 Gy, against 37.5% in the control population.

For Series II, chronic irradiation was performed until TD reached 4 Gy (DR=10 cGy/d), employing 102 animals and

126 controls. Stem cell distribution (SCD) parameters were evaluated at 2 d (TD = 4 Gy), 6 and 12 mo (after TD of 2 or 4 Gy), and at 18 mo (after TD of 2.0 Gy). Results are presented in table 8.

Significant rearrangement of SCD was found as early as 2 d after chronic irradiation with 4 Gy, that is, the percentage of cells in the DNA-presynthetic phase was decreased, whereas the ratio of the $S+G_2+M$ subpopulation increased ($p < 0.01$). This peculiarity of SCD was maintained at 6, 12 (TD = 2 or 4 Gy), and 18 mo (TD = 2 Gy) due to the increased fraction of DNA-synthesizing (S phase) cells. Such longitudinal increase in cycling ($S+G_2+M$) cells was rather pronounced, thus far exceeding both circadian (yearly) changes and the age-associated increase of this parameter assessed for untreated animals.

The effects of chronic irradiation (TD = 4 Gy, Series I and II) were compared for experiments performed at different seasons (spring and autumn), corresponding, respectively, to minimal and maximal amounts of $S+G_2+M$ marrow cells in control animals. This design permitted us to compare the extent of postradiation hemopoietic damage when induced at various seasons (table 8). In both Series I and II, a significant increase in $S+G_2+M$ cells was found ($p < 0.01$). However, 2 d after spring irradiation was completed, these changes were determined, mainly, by increased percentages of G_2+M myelokaryocytes ($p < 0.001$). Meanwhile, the immediate changes in marrow SCD after autumn trials were characterized, primarily, by an increased fraction of S-phase myelokaryocytes ($p < 0.001$).

Six months after the termination of treatment, an increased percentage of DNA-synthesizing marrow cells was detected in irradiated rats from both series, compared to untreated age-matched controls ($p < 0.05$). For Series I and II, this parameter was increased to 161 ± 28 ($p < 0.05$) and $130 \pm 12\%$ ($p < 0.01$), respectively, of control values.

The mentioned seasonal differences in radiation responses may be explained by season-dependent marrow SCD specificities, causing variability in marrow proliferation dynamics. Under these circumstances, the radiation factor and circadian changes of the hemopoietic system may interact in either an additive or antagonistic manner.

In separate experiments, additional acute irradiation at a challenge dose of 1 Gy was performed 12 mo after chronic radiation treatment. Two days after test treatment, SCD was evaluated in experimental animals and age-matched controls.

The bone marrow from control rats exhibited a marked increase in S-phase cells: (15.04.90 - 23.04.90) from 10.6 ± 0.7 to $14.3 \pm 1.7\%$ ($p < 0.05$), and (01.06.90 - 13.06.90) from 7.7 ± 0.6 to $10.2 \pm 0.8\%$ ($p < 0.05$), along with a decreased percentage of G_0+G_1 myelokaryocytes: (15.04.90 - 23.04.90) from $80.9 + 0.8$ to $75.8 \pm 1.9\%$ ($p < 0.05$), and (01.06.90 - 13.06.90) from 84.0 ± 0.8 to $81.7 \pm 0.9\%$ ($p > 0.05$).

In contrast, only marginal hemopoietic responses to test irradiation were detected for previously irradiated animals (12 mo after the end of chronic treatment at TD of 2 or 4 Gy, DR = 0.625 cGy/sec). These rats did not exhibit any marked changes in SCD ($p > 0.05$) 2 d after 1 Gy of acute irradiation. However, it is worth noting that a similar lack of postradiation response was found in rats that survived acute radiation syndrome. Hence, this functional pathology may possibly result from radiation damage to relevant hemopoietic progenitors. Therefore, more detailed studies of deficient responses, as revealed by SCD analysis, were performed with respect to their dose- and dose-rate dependence.

Dose Dependence of Longitudinal SCD Changes

Dose dependence of bone marrow cell-cycle changes was studied at different times after chronic gamma irradiation performed at a constant dose rate (14 cGy/day). The TDs applied were 0.12, 0.28, 0.54, 1.00, and 2.00 Gy. A total of 340 rats was examined. SCD was analyzed at 1, 6, and 12 mo after the end of irradiation. The results of this study are presented in table 9.

Data obtained at 1 mo after treatment indicated a distinct rearrangement of cell populations with regard to their cycling parameters. An increased percentage of DNA-synthesizing cells was found after treatment with TDs of 0.54 ($p < 0.01$), 1.00, and 2.00 Gy ($p < 0.001$), along with decreased amounts of $G_0 + G_1 + M$ myelokaryocytes after irradiation with 0.28, 0.54 ($p < 0.01$), 1.0, and 2.0 Gy ($p < 0.001$). At 6 mo, the magnitude of these alterations decreased gradually. Relevant differences between controls and irradiated animals became marginal after TDs of 0.12 and 0.28 Gy ($p > 0.05$), while remaining altered at higher TDs. At 12 mo, the differences for these parameters ($p < 0.01$) persisted only after the highest dose applied (2.0 Gy).

Table 8. Bone marrow cell cycle changes after irradiation of rats

Day, month, year	N of rats	Period (mo)	Total dose Gy	Cell percentage G_0 / G_1	S	G_2 / M
			Dose rate = 5 cGy/d			
15.04.89	17	2 d	4	86.7 ± 0.4	5.5 ± 0.6	7.7 ± 0.3
(c)	13			89.7 ± 0.3	4.8 ± 0.5	5.5 ± 0.2
				p < 0.001	p > 0.05	p < 0.001
02.11.89	20	6	4	77.8 ± 2.1	10.8 ± 1.4	11.4 ± 0.9
(c)	8			84.1 ± 0.5	6.7 ± 0.8	9.2 ± 0.5
				p < 0.01	p < 0.05	p > 0.05
10.12.89	20	6	2	74.8 ± 1.1	14.2 ± 1.1	11.0 ± 0.4
(c)	8			81.6 ± 1.2	8.5 ± 1.5	9.9 ± 0.6
				p < 0.001	p < 0.01	p > 0.05
15.04.90	76	12	4	75.8 ± 0.7	14.6 ± 0.5	9.7 ± 0.3
(c)	40			80.9 ± 0.8	10.6 ± 0.7	8.7 ± 0.4
				p < 0.001	p < 0.001	p < 0.05
01.06.90	22	12	2	80.8 ± 0.9	11.9 ± 0.7	7.3 ± 0.7
(c)	9			84.0 ± 0.8	7.7 ± 0.6	8.3 ± 0.6
				p < 0.05	p < 0.001	p > 0.05
17.01.91	11	18	2	76.3 ± 1.3	12.5 ± 0.8	10.7 ± 0.8
(c)	9			81.2 ± 1.0	9.5 ± 0.9	9.4 ± 0.5
				p < 0.01	p < 0.05	p > 0.05
			Challenge dose = 1 Gy at 12 mo after initial treatment			
23.04.90	16	2 d	4 (+ 1)	72.1 ± 1.7	17.8 ± 1.6	10.1 ± 0.6
(c)	8			75.8 ± 1.9	14.3 ± 1.7	9.9 ± 0.8
				p > 0.05	p > 0.05	p > 0.05
13.06.90	16	2 d	2 (+ 1)	80.2 ± 1.0	10.8 ± 0.9	7.0 ± 0.7
(c)	8			81.7 ± 0.9	10.2 ± 0.8	8.8 ± 0.9
				p > 0.05	p > 0.05	p > 0.05
			Dose rate = 10 cGy/d			
11.10.92	10	2 d	4	69.6 ± 1.5	18.8 ± 1.3	11.6 ± 0.5
(c)	10			75.3 ± 1.0	11.5 ± 0.8	13.2 ± 0.7
				p < 0.01	p < 0.001	p > 0.05
30.03.93	16	6	4	79.0 ± 0.6	13.0 ± 0.4	8.0 ± 0.4
(c)	8			81.9 ± 0.7	10.0 ± 0.9	8.1 ± 0.8
				p < 0.01	p < 0.01	p > 0.05

(c) = controls

Table 9. Bone marrow cell cycle changes after different total doses of chronic irradiation (DR = 14 cGy/d)

Day, month, year	N of rats	Total dose Gy	Cell percentage		
			G_0/G_1	S	G_2/M
1 month after irradiation					
20.04.92	20	0.12	81.0 ± 1.1 p > 0.05	10.3 ± 1.0 p > 0.05	8.7 ± 0.4 p > 0.05
20.04.92	19	0.28	79.3 ± 1.2 p < 0.01	11.0 ± 1.3 p > 0.05	9.7 ± 0.4 p < 0.05
20.04.92	20	0.54	79.9 ± 0.9 p < 0.01	11.7 ± 0.9 p < 0.01	8.4 ± 0.4 p > 0.05
20.04.92	19	1.00	77.5 ± 0.4 p < 0.001	13.1 ± 0.5 p < 0.001	9.4 ± 0.5 p < 0.05
20.04.92	28	2.00	76.0 ± 1.4 p < 0.001	16.0 ± 1.4 p < 0.001	8.0 ± 0.5 p > 0.05
(c)	42		83.0 ± 0.5	8.6 ± 0.4	8.4 ± 0.3
6 months after irradiation					
09.09.92	10	0.12	80.3 ± 0.4 p > 0.05	10.6 ± 0.5 p > 0.05	9.1 ± 0.4 p > 0.05
09.09.92	10	0.28	80.8 ± 0.8 p > 0.05	10.3 ± 0.9 p > 0.05	8.9 ± 0.6 p > 0.05
09.09.92	10	0.54	77.9 ± 1.1 p < 0.05	12.1 ± 0.9 p < 0.05	10.0 ± 0.6 p > 0.05
09.09.92	10	1.00	78.3 ± 0.6 p < 0.01	12.7 ± 0.6 p < 0.001	9.0 ± 0.4 p > 0.05
09.09.92	10	2.00	75.8 ± 1.2 p < 0.01	15.2 ± 1.0 p < 0.001	8.9 ± 0.7 p > 0.05
(c)	10		80.5 ± 0.5	10.1 ± 0.3	9.4 ± 0.3
12 months after irradiation					
01.03.93	7	0.12	83.8 ± 0.6 p > 0.05	9.2 ± 0.7 p > 0.05	7.0 ± 0.2 p > 0.05
01.03.93	7	0.28	81.7 ± 0.3 p > 0.05	10.1 ± 0.3 p > 0.05	8.2 ± 0.3 p > 0.05
01.03.93	8	0.54	81.5 ± 0.5 p > 0.05	10.4 ± 0.4 p > 0.05	8.1 ± 0.4 p > 0.05
01.03.93	8	1.00	81.4 ± 0.3 p > 0.05	10.8 ± 0.6 p > 0.05	7.8 ± 0.5 p > 0.05
01.03.93	8	2.00	80.4 ± 0.9 p > 0.05	12.5 ± 0.7 p < 0.01	7.1 ± 0.5 p > 0.05
(c)	10		82.5 ± 0.7	9.7 ± 0.4	7.8 ± 0.5

(c) = controls

Recovery Trends of DNA-Synthesizing Cell Populations

In order to exclude interference of this pathology with circadian and age-associated SCD changes, the parameters were normalized as irradiation/control ratios (Si/Sc). Both irradiated rats and their age-matched untreated counterparts were examined simultaneously. Experimental animals treated with TDs of 2 Gy (DR = 5 cGy/d) were evaluated 6, 12, and 18 mo after the end of irradiation (table 8).

This approach again revealed a relatively increased percentage of DNA-synthesizing myelokaryocytes 6 mo after the completion of prolonged irradiation. The differences from controls became less pronounced upon aging of animals (at 12 and 18 mo).

Regression analysis of this dynamic has shown that the relative contents of DNA-synthesizing cells decreases with time according to the following equation:

$$Si/Sc = 1.86 - 0.03t$$

where t is the time (mo) elapsed since the completion of chronic irradiation.

Hence, the percentage of DNA-synthesizing myelokaryocytes in rats surviving chronic irradiation of 2.0 Gy would reach control levels at about 2.5 years (29 mo) after the end of treatment. This is well beyond the mean life expectancy for rats maintained under laboratory conditions.

Recovery of Immune Cell Populations

The reconstitution of lymphoid tissues following chronic irradiation proceeds in a phasic manner [42,49], that is, the periods of hyperregeneration and late hypoplasia are known for thymus and spleen populations. In murine experiments, the number of Lyt-1 and Lyt-2 cells experienced irregular time-dependent changes. However, clear connections were found between late postradiation deficiency of hemopoietic stem cells, later lymphoid hypoplasia, and long-term impairment of functional B-cell populations in chronically irradiated mice [50].

Internal irradiation of animals (a model with tritiated water incorporation) exerted much more severe and prolonged immune depression compared to external irradiation at the same total doses [27,42,50], thus showing

higher relative biological effectiveness values of incorporated radionuclides.

Genetic Abnormalities

Chromosome Aberrations

Chromosome pathology, as assessed by anaphase analysis, was found in dogs subjected to LDR irradiation [3]. At 0.17 - 0.34 cGy/d, the levels of aberrant cells increased from the first year of the experiment, presenting, mainly, with chromatid-type aberrations and occasional bridges with acentric fragments. In some experiments, clonal hemopoiesis was revealed in bone marrow, as shown by atypical marker chromosomes [51]. Clonality of hemopoiesis may be combined with symmetric chromosome exchanges [35].

Micronuclei

The incidence of micronuclei (MN) is a widely used marker of general genomic pathology, including radiation-induced lesions. Increased incidence of Jolly bodies (the micronuclei, as seen in erythrocytes) was previously found in chronically irradiated dogs [3]. RBCs with Jolly bodies were frequently found in chronic rat experiments [1].

Moreover, micronuclear counts could be used as a marker of adaptive response induced by prolonged irradiation [52]. Acute challenge irradiation revealed much weaker MN induction in chronically treated mice than in nonirradiated controls. Hence, murine marrow cells surviving chronic treatment proved to be more radioresistant, thus being in accord with data on GM-CFU radioresistance in chronically irradiated dogs [7,8].

Point Mutations

Russian literature is lacking studies of point mutations for detecting blood pathology in chronically irradiated animals (e.g., inactivation of HGPRT, TK, glycophorin). However, human data show the applicability of these approaches, at least at higher cumulated doses [53].

Combined Action of Chronic Irradiation and Heavy Metal Ions

Under real environmental conditions, radiation exposure may be accompanied by exposure to toxic chemicals, thus necessitating appropriate experimental and human studies [54]. Among environmental toxicants, health effects of certain heavy metals are of special importance, especially those of lead, mercury, cadmium, zinc, and chromium [55]. The toxicity of individual metal compounds depends on their ingestion rates, tissue distribution, and mechanisms of interference with basic biochemical events in living cells. For example, some metal ions (chromium, copper) induce DNA breaks, thus exerting radiation-like damage. Cadmium is likely to produce strong nephrotoxic and neurotoxic effects, whereas lead, a known hemodepressive agent, interferes with hemoglobin synthesis [56].

Therefore, the hematological effects of two metals (lead and cadmium) and low dose/low dose rate ionizing irradiation (TD = 4 Gy) were studied in our experiments [20,21]. The separate effects of both metal salts were compared to combined actions of metal/chronic irradiation. Chemical toxicants were given with drinking water at clearly toxic doses, corresponding to the tenfold maximal allowed concentrations (MAC) established in Russia.

A total of 651 rats was involved in these experiments. The following exposures were studied for various groups of animals:

1. None (untreated controls), n = 126
2. Chronic irradiation (TD = 4 Gy; DR = 2.4 μGy/sec; n = 102)
3. Cadmium chloride administration (5 mg/l drinking water for 40 days, n = 97)
4. Lead acetate administration (50 mg/l water for 40 days, n = 114)
5. Chronic irradiation (see group 2) plus cadmium treatment (see group 3), n = 104
6. Chronic irradiation (see group 2) plus lead administration (see group 4), n = 108.

Peripheral blood counts were performed for all groups of animals. Briefly, immediate differences were not obvious between experimental groups and control animals with respect to RBC and platelet counts, total numbers of leukocytes and their differential counts, hemoglobin, and erythrocyte sedimentation rate values. Only a trend to leukocytosis was revealed in animals treated with lead salt as well as decreased reticulocyte numbers for animals subjected to combined irradiation/lead exposure.

At 6 mo after completion of treatment, the peripheral blood indexes were, generally, within normal limits. However, we found increased reticulocyte counts in lead-treated animals as compared to normal reticulocyte contents after radiation/lead treatment (214,000 ± 18,000 and 96,000 ± 23,000/μL, respectively). These alterations were not observed at 12 months after treatment. Meanwhile, WBC differential counts did not show any peculiarities in lead- or cadmium-exposed groups of rats at later times.

The average sizes of lymphocyte nuclei in controls and immediately after completion of chronic irradiation were 7.31 ± 0.76 μm and 6.96 ± 0.12 μm, respectively, thus showing no significant effects of radiation exposure. Similarly, the diameter of cellular nuclei was not affected after cadmium or lead treatment (6.70 ± 0.51 μm and 6.69 ± 0.32 μm, respectively). Likewise, no evidence for combined effects of radiation and metals upon this parameter was found.

Blood leukocyte adherence and phagocytic properties of granulocytes were not affected by treatment with cadmium alone or by the combined radiation/metal action.

Differential counts of mature myeloid cells in marrow smears proved to be unchanged soon after irradiation (table 3). Meanwhile, the ratios of young myeloid cells were decreased in irradiated and cadmium-treated rats. These changes, however, did not persist at 2 months after completion of treatment.

The most pronounced alterations of the bone marrow pattern were found in animals exposed to lead or lead + irradiation. In these groups, a sharp decrease of proliferating cell pools was noted, both immediately after exposure and 2 mo later. Total numbers of young and maturating marrow cells were also diminished after lead treatment followed, however, by recovery at 2 mo.

The erythroid population was found to be expanded after lead or lead + irradiation exposures (as well as after irradiation alone). Following lead treatment, this effect lasted for at least 2 mo, whereas it did not persist this long after lead + irradiation or irradiation only treatment. For cadmium- or cadmium + irradiation-treated groups, no immediate changes in this population were noted (table 10). This finding correlates well with late reticulocytosis in peripheral blood.

On the contrary, the effects of lead (group 4) manifested as decreased percentages of both S and G_2+M myelokaryocytes ($p < 0.01$), with the effects of radiation and lead (group 6) apparently antagonistic, since no changes in cell-cycle parameters were found when compared to the concomitant increase in G_0/G_1 populations ($p < 0.001$).

The group-specific characteristics of marrow pathology persisted from 2 to 12 mo in experimental groups subjected to irradiation, cadmium, or both toxic factors. Meanwhile, the animals fed with lead acetate exhibited gradual recovery of marrow depression, as evidenced by the reversal of SCD to control values at 2 mo. After combined radiation/lead treatment, the long-term effects of radiation seemed to become predominant at this time, thus producing a moderate increase in DNA-synthesizing marrow cells ($p < 0.01$). However, this effect was not observed at 12 mo, suggesting

Table 10. Cellular composition of bone marrow immediately after chronic irradiation (TD = 4 Gy) and/or heavy metal treatment

Type of cells	Treatment					
	Control	Cd	Cd+Rad	Lead	Lead+Rad	Rad
Myeloblasts, promyelocytes, and myelocytes	10.6 ± 0.6	8.3 ± 2.4	7.8 ± 2.0	4.1 ± 0.5	3.4 ± 0.3	7.6 ± 2.1
Metamyelocytes, neutrophils	46.1 ± 2.1	44.6 ± 1.2	41.2 ± 2.0	38.6 ± 1.8	40.1 ± 2.5	42.3 ± 2.3
Erythroblasts, normoblasts	10.9 ± 1.2	10.3 ± 4.4	11.9 ± 2.7	15.4 ± 1.1	16.2 ± 1.2	16.8 ± 1.3
Lymphocytes + plasmatic cells	27.9 ± 2.2	31.7 ± 5.2	32.8 ± 3.9	35.1 ± 2.5	32.2 ± 2.3	29.7 ± 4.7
Eosinophils	5.6 ± 0.6	5.3 ± 0.2	7.1 ± 0.8	7.2 ± 0.6	7.5 ± 0.8	5.3 ± 0.9

CCD Disturbances of Rat Myelokaryocytes

The results of bone marrow SCD studies are presented in table 11. Two days after completion of treatment, the cell-cycle parameters of myelokaryocytes differed widely among the experimental groups. Following single radiation exposure (group 2), the number of DNA-synthesizing cells was sharply increased ($p < 0.001$), along with diminished contents of G_0/G_1 cells ($p < 0.01$). Similar changes were detected in the radiation + Cd group, whereas Cd administration alone did not induce any changes in these indexes.

a severe pathology of hemopoiesis induced by the lead exposure. An increase in S-phase myelokaryocytes ($p < 0.01$), which may be attributed to the radiation factor, was also found at 12 mo after combined radiation/cadmium treatment.

Some conclusions on this experimental study may be drawn, as follows:

- LDR chronic irradiation of experimental animals causes substantial activation of different bone marrow

Table 11. Changes of bone marrow cell cycle distribution after combined chronic gamma irradiation of rats at a 4.0 Gy dose (dose rate = 10 cGy/d) and/or heavy metal ions (Cd and Pb)

Date of study	N	Factor	Percentage of cells in various phases					
			G_0/G_1		S		G_2/M	
2 days after the completion of treatment								
16-22.09.92	30	4 Gy + Pb	77.4 ± 0.8	p > 0.05	13.2 ± 0.7	p > 0.05	9.4 ± 0.4	p > 0.05
11-16.09.92	30	Pb	82.8 ± 0.7	p < 0.001	7.8 ± 0.5	p < 0.001	9.4 ± 0.5	p < 0.01
16-22.09.92	10	4 Gy + Cd	70.1 ± 2.0	p < 0.05	17.9 ± 1.6	p < 0.01	12.0 ± 1.1	p > 0.05
11-16.09.92	10	Cd	75.0 ± 1.3	p > 0.05	11.1 ± 1.4	p > 0.05	13.9 ± 0.8	p > 0.05
16-22.09.92	10	4 Gy	69.6 ± 1.5	p < 0.01	18.8 ± 1.3	p < 0.001	11.6 ± 0.5	p > 0.05
11-16.09.92	10	Control	75.3 ± 1.0		11.5 ± 0.8		13.2 ± 0.7	
2 - 2.5 months after the completion of treatment								
16-22.11.92	33	4 Gy + Pb	80.2 ± 0.3	p > 0.05	11.5 ± 0.3	p < 0.01	8.3 ± 0.3	p < 0.05
12-16.11.92	27	Pb	81.6 ± 0.3	p > 0.05	10.0 ± 0.3	p > 0.05	8.4 ± 0.3	p < 0.05
16-22.11.92	10	4 Gy ± Cd	81.1 ± 0.6	p < 0.001	8.9 ± 0.6	p < 0.05	10.0 ± 0.7	p < 0.05
12-16.11.92	10	Cd	76.0 ± 1.1	p > 0.05	11.4 ± 0.9	p > 0.05	12.6 ± 0.9	p > 0.05
16-22.11.92	10	4 Gy	75.9 ± 1.1	p < 0.001	12.1 ± 0.6	p < 0.01	12.0 ± 0.9	p > 0.05
12-16.11.92	10	Control	79.4 ± 1.1		10.3 ± 0.7		10.3 ± 0.9	
6 months after the completion of treatment								
07.04.93	10	4 Gy + Pb	82.6 ± 0.7	p > 0.05	10.5 ± 0.5	p > 0.05	6.9 ± 0.6	p > 0.05
05.04.93	10	Pb	84.0 ± 0.8	p > 0.05	7.8 ± 0.8	p > 0.05	8.3 ± 0.5	p > 0.05
09.04.93	10	4 Gy + Cd	80.3 ± 1.2	p > 0.05	13.6 ± 0.8	p < 0.01	6.1 ± 0.5	p < 0.05
08.04.93	10	Cd	81.8 ± 0.8	p > 0.05	10.3 ± 0.6	p > 0.05	7.9 ± 0.4	p > 0.05
30.03.93	16	4 Gy	79.0 ± 0.6	p < 0.01	13.0 ± 0.4	p < 0.01	8.0 ± 0.4	p > 0.05
07.04.93	8	Control	81.9 ± 0.7		10.0 ± 0.9		8.1 ± 0.8	
12 months after the completion of treatment								
11.10.93	10	4 Gy + Pb	76.9 ± 0.8	p > 0.05	12.6 ± 0.6	p > 0.05	10.5 ± 0.5	p > 0.05
12.10.93	10	Pb	79.4 ± 0.7	p > 0.05	10.7 ± 0.6	p > 0.05	9.9 ± 0.5	p > 0.05
13.10.93	10	4 Gy + Cd	77.5 ± 0.6	p > 0.05	12.9 ± 0.3	p < 0.01	9.8 ± 0.5	p > 0.05
14.10.93	9	Cd	79.5 ± 0.8	p > 0.05	10.6 ± 0.6	p > 0.05	9.8 ± 0.5	p > 0.05
15.10.93	8	Control	79.0 ± 0.8		11.3 ± 0.4		9.7 ± 0.4	

lineages, thus producing a stable increase in S-phase hemopoietic cell populations.

- These radiation-induced changes in cell cycle distribution greatly exceed seasonal or age-dependent trends.
- Toxic metal ions (e.g., lead), if applied in clearly toxic doses, may produce a time-dependent depressive effect upon bone marrow cytokinetics.
- Combined chronic treatment with both LDR radiation and toxic metal reveals an antagonistic action, as evidenced by DNA flow cytometry and morphological studies of bone marrow samples.
- The radiation effect predominated over a longer time after combined radiation/metal treatment.
- Our limited study shows that radiomodifying effects of various metals and other toxic chemicals should be taken into account when arranging appropriate safety regulations.

General Conclusions

- Hemopoietic stem cells and blood cell progenitors seem to be the main target of chronic LD/LDR irradiation.
- Direct radiation damage to the blood/immune precursor pool results in a decrease of stem cell fraction.
- Radiation-induced depletion of the stem cell and progenitor pool results in accelerated cycling of bone marrow precursors.
- Decreased viability of mature blood cells results from ineffective hemopoiesis, thus causing restriction of myeloid (and, probably, lymphoid) cell reserves.
- Disturbances of cellular and humoral immunity are likely to be caused by extreme radiosensitivity of lymphoid tissues and by a restricted progenitor pool.
- Postirradiation recovery is characterized by gradual reconstitution of peripheral blood and bone marrow patterns. However, partial deficiency of hemopoietic and lymphopoietic precursors may be a limiting factor in blood/immune system recovery.
- An increased percentage of S-phase marrow cells well after prolonged radiation treatment presents a typical response of the hemopoietic system to low dose rate irradiation, since it is not observed after acute or subacute irradiation or following chronic exposure to some heavy metal ions.
- DNA misrepair following chronic irradiation may present with stable chromosome aberrations, increased incidence of micronuclei, and detectable point mutations of blood/immune populations.
- The radiation-induced carcinogenic potential could be expressed as one of the following:
  - Clonal hematopoiesis with marker chromosomes revealed
  - Preleukemia-like bone marrow changes
  - Evolving lymphoproliferative or myeloproliferative disease.
- Simultaneous recording of bone marrow cellularity and cell cycle distribution should provide more precise bioassessment of radiation pathology. These parameters may be especially useful as biological indicators of radiation damage induced at low dose rates, both immediately and well after exposure.

Informative Blood/Immune System Parameters

According to Russian studies conducted from 1972 through 1995, the following parameters are particularly informative after low dose/low dose rate irradiation in animal experiments.

- Peripheral blood indexes:
  - Reticulocyte counts
- Bone marrow:
  - Differential counts of marrow samples
  - Parameters of cell-cycle distributions in myeloid and erythroid compartments
  - Marrow reserves of mature granulocytes and RBCs
  - Relative contents of micronuclei in polychromatophilic erythrocytes.
- Immune cell populations:
  - T-lymphocyte cultures for chromosome analysis
  - *In vitro* response of lymphoid cells to T- and B-cell mitogens

- Cellularity of spleen and thymus
- Plaque-forming cell counts in spleen after *in vivo* immunization
- Rates of cytokine (e.g., interleukins) production by lymphoid cells and macrophages
- Contents of antimicrobial and, especially, autoantibodies in blood serum

Possible Implications for Human Studies

Potential Cohorts for Long-Term Studies of Chronic Radiation Effects

- Miners exposed to radon and other fission products
- Nuclear fuel operators
- Medical radiologists
- Staff of nuclear submarines and spacecrafts
- Accidentally exposed cohorts (e.g., Chernobyl victims)

Dosimetric Problems in Accidentally Exposed Cohorts

Exact radiation dosimetry in humans is a challenge to radiologists, in contrast to well-planned animal experiments. The problem of reconstructing individual doses in exposed cohorts has been with us since our early experience with Hiroshima and Nagasaki victims. The Chernobyl disaster posed numerous problems with the evaluation of radiation doses received from both external and internal sources. Hence, the magnitude of doses absorbed by hemopoietic tissues is largely undetermined under real field conditions. These issues are especially important when discussing long-term health effects of mixed beta- and gamma-emitting isotopes with different lifetimes.

The true radiation doses in each case of human exposure should be estimated with great caution, even when the data from personal dosimeters are available. The uncertainty about physical dosimetry arises largely from common flaws in this approach during radiation accidents. Even in the presence of personal radiation monitors, the exposure dose is registered only at a single site on the body. Hence, a true dose distribution throughout the hemopoietic and immune system should be reconstructed in every case of accidental irradiation.

When dealing with the aftereffects of LD/LDR irradiations, we should expect rather weak expression (incidence) of pathobiological effects in blood and immune cells. These lesions should be expressed at the same order of magnitude as other common genotoxic factors present in the environment (e.g., cancerogenic chemicals, toxic metal ions).

Individual radiosensitivity of cells and tissues is another factor that strongly limits the precision of biological dosimetry, especially at low doses of irradiation. Postradiation recovery and repair quality depends on DNA repair capacity, thus determining the rates of mutagenesis/carcinogenesis in the target tissue. Moreover, recent data point to particular damage from low dose irradiation, especially membrane damage and mismatched DNA repair.

The uncertainty of low-level radiation effects and background environmental contamination makes it necessary to detect the summary effects of harmful genotoxic factors. Blood and immune cell populations are very well suited for such molecular dosimetry.

Possible Approaches to Human Studies of Blood/Immune Pathology

- Sources of biological material:
  - Peripheral blood cells, as fractionated lymphocytes, PMNs, and RBCs
  - Bone marrow biopsies (in cases of evident blood disorders)
  - Peripheral blood stem cells (PBSC) mobilized in exposed persons' recombinant growth factors (by informed consent)
- Peripheral blood parameters:
  - Routine blood cell counts (limited use)
  - Reticulocyte numbers, including counts of micronuclei

- Differential counts of marrow cells.

- Hematopoietic cell studies (with bone marrow or PBSC):
  - Self-renewal potential (CFU-mix numbers)
  - Cell cycle distribution studies (S-phase cell fraction)
  - Commitment of precursors (CFU-C, BFU-E numbers)

- Committed precursors
  - Proliferation rates of CFU-GM
  - Expansion of RBC precursors (CFU-Er, proliferative index of erythroblasts)
  - Presence and density of specific cytokine receptors on precursor cells
  - S-phase compartment of bone marrow (DNA flow cytometry)

- Immune cell populations
  - Studies of functional maturation of marrow myeloid cells (phagocytosis, metabolic burst, expression of adherence capacity, integrin and other adherence molecules, and bactericidal proteins)
  - Essential characteristics of peripheral lymphocytes (T- and B-cell counts; CD4+/CD8+ ratios) by means of cytotoxic assays or FACS technique

- Radiation sensitivity assays *in vitro*, using clonogenic blood progenitors and peripheral lymphoid cells
  - Dose-response curves (colony assays, apoptosis counts)
  - Assessment of adaptive response and cellular radioresistance
  - Rates of DNA damage and repair caused by *in vitro* UV or gamma irradiation (comet assay)

- Genotoxic assays:
  - Rates of stable chromosome aberrations in lymphoid and marrow cultures, employing FISH analysis with appropriate DNA probes
  - Assessment of micronuclei in lymphocyte cultures
  - Detection of some point mutations (HGPRT, TK, etc.) using long-term cultures of blood cells

- Molecular biology studies (in context of immune studies and assessment of carcinogenic potential):
  - Expression of several oncogenes, p53 anti-oncogene (wild and mutant genotypes), by *in situ* hybridization
  - Spontaneous apoptotic rates, along with APO/Fas (CD95 antigen) expression on the surface of mononuclears
  - Expression of GM-CSF, IL-3, M-CSF, IL-2, IL-4, IL-5, and interferon gamma as studied in mononuclear blood cells, using mRNA *in situ* hybridization and immunostaining.

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13. ABSTRACT *(Maximum 200 words)*

The interaction of low dose/low dose rate radiation with heavy metals (in this report cadmium and lead) is important for at least two reasons: 1) radiation workers and populations exposed to unusually high background levels of radiation (e.g. Chernobyl and the Techa river in Russia) receive this type of exposure rather than the much better studied prompt high dose exposure, and 2) radiation accidents frequently involve the release of other contaminants as well. Even at low radiation doses and dose rates, the effects of simultaneous or near simultaneous exposure to cadmium or lead, which are marrow toxicants in their own right, are additive or synergistic to those of radiation.

Although there is an adaptive response to low dose rate exposures, recovery of marrow precursor cells after a second exposure may be incomplete. Detection of damage repair changes after low dose exposures is difficult. The authors describe possible mechanisms for the observed delayed or incomplete recovery. They also address the complex pathogenic changes seen when heavy metals are introduced along with the radiation.

The implications of their findings in rodent models for human populations are discussed, and the importance and proposed directions of further work in this field described.

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